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# Intraoral electric potential *via* oral bacterial power generation —A novel mechanism of biofilm formation

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In the early stages of biofilm accumulation, the electric charge of the dental enamel and pellicle surfaces is known to be involved. We therefore investigated the relationship between oral hygiene and intraoral electric potential (IoP) in 45 male participants using a double-blind study. IoP, but not body surface electric potential, was loosely correlated with oral hygiene condition (Oral Hygiene Index; OHI). IoP was also loosely correlated with smartphone use; however, there was no significant correlation between smartphone use and OHI. IoP elevation might be caused by OHI elevation resulting from biofilm formation as an internal factor, with smartphone use as an external factor. This *in vitro* study revealed the generating capacity of *Streptococcus mutans* accompanied by biofilm accumulation using a microbial fuel cell. These results suggest that IoP elevation is caused by biofilm accumulation induced by power generation of oral bacteria, resulting in elevation of OHI.

*Keywords*: Oral health examination, Questionnaire survey, Microbial fuel cell, *Streptococcus mutans*, Biofilm accumulation

## INTRODUCTION

The mouth is a mirror of the body. Many systemic diseases are characterized by symptoms that appear in the oral cavity[1,2]. Oral bacteria, including those associated with dental caries and periodontal disease, are known to be a risk factor for certain systemic diseases, such as infective endocarditis, atheromatous plaques, diabetes, pre-eclampsia, pneumonia, obesity, cancer, and cognitive impairment[3-5]. Knowledge of the oral condition is an important component of knowledge of the health of the whole body. Static electricity collected in the human body could influence health and daily life[6]. Release of static electricity or "earthing" is reported to be beneficial to health[7,8].

Oral bacterial flora adhere to the electrically charged surfaces of the dental enamel and pellicle *via* divalent cations (such as $Ca^{2+}$) in the saliva[9-11]. This mechanism contributes to biofilm formation, especially in the early stages, and is thus important in oral hygiene and health. Poor oral hygiene causes dental caries and periodontal disease, and ultimately leads to the loss of teeth. Tooth loss leads not only to impaired mastication and deglutition, but also to a decline in physical and cognitive function[12]. Mastication plays an important role in normal growth of the jaw and dental arch, saliva secretion, digestion and immunity, and health maintenance[13]. Maintenance of good oral hygiene is an important and indispensable factor for improving quality of life and extending healthy life expectancy.

The aim of this study was therefore to investigate the relationship between human electric potential (intraoral electric potential [IoP] and body surface electric potential [BsP]) and oral hygiene/health in 45 male participants in a double-blind study, and the relationship between power generating capacity and biofilm formation in oral bacteria using a microbial fuel cell (MFC) in an *in vitro* study.

## MATERIALS AND METHODS

We performed an *in vivo* study of oral health in accordance with the STROBE recommendations[14], following the recommendations from the Ethics Committee of the Research Ethics Committee of the Nippon Dental University School of Life Dentistry at Niigata (Ethical approval number; ECNG-H-267) and the Declaration of Helsinki[15].

### *In vivo study design and participants*

Participants of this study were 45 male volunteers with no systemic diseases and no missing teeth who were fifth year university students (mean age; 20.1±0.96 years, range; 19.3–23.7 years) at the National Institute of Technology, Nagaoka College, who consented to participate after detailed explanation of the study

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procedure. Female students were not selected as participants because of the possible effects of hormonal fluctuations on oral conditions including oral hygiene and periodontal status. The participants completed a questionnaire survey about their daily life habits, including time spent sleeping; eating; smoking; using a personal computer, television and smartphone; and toothbrushing. Written consent was obtained in all cases before commencement of the study. The participants were randomly divided into three groups, and each group (15 participants) underwent a range of measurements at the same time (15:00–17:00) each day for 3 days under the same measurement conditions (the same room at a temperature of 24.6±0.50°C [range; 24.1–25.1°C] and humidity of 67.7±5.69% [range; 63–74%]).

One day before the measurements were taken, participants were asked to control their intake of food and drink, and their use of fragrances, *i.e.* to limit their intake/application of strong-smelling substances and breath fresheners. On the day of the measurement, participants were asked to limit tooth brushing, dietary intake, breath freshener use and smoking after lunch.

*Measurement of electric potentials, oral condition and vital signs*

The measurements were undertaken in the order shown in Fig. 1. All measurements were performed noninvasively.

In accordance with the JIS standard[16] and the IEC standard[17], IoP/BsP values were estimated using a digital multimeter (DMM) (7351A/E, ADC, Tokyo, Japan) with a sterilized electrode consisting of a 5 cm stainless steel wire for orthodontic appliances (0.90 mm in diameter, made of SUS304, Tomy International, Tokyo, Japan) (Fig. 1). The validity of the IoP/BsP estimation was confirmed by directly grounding the oral/

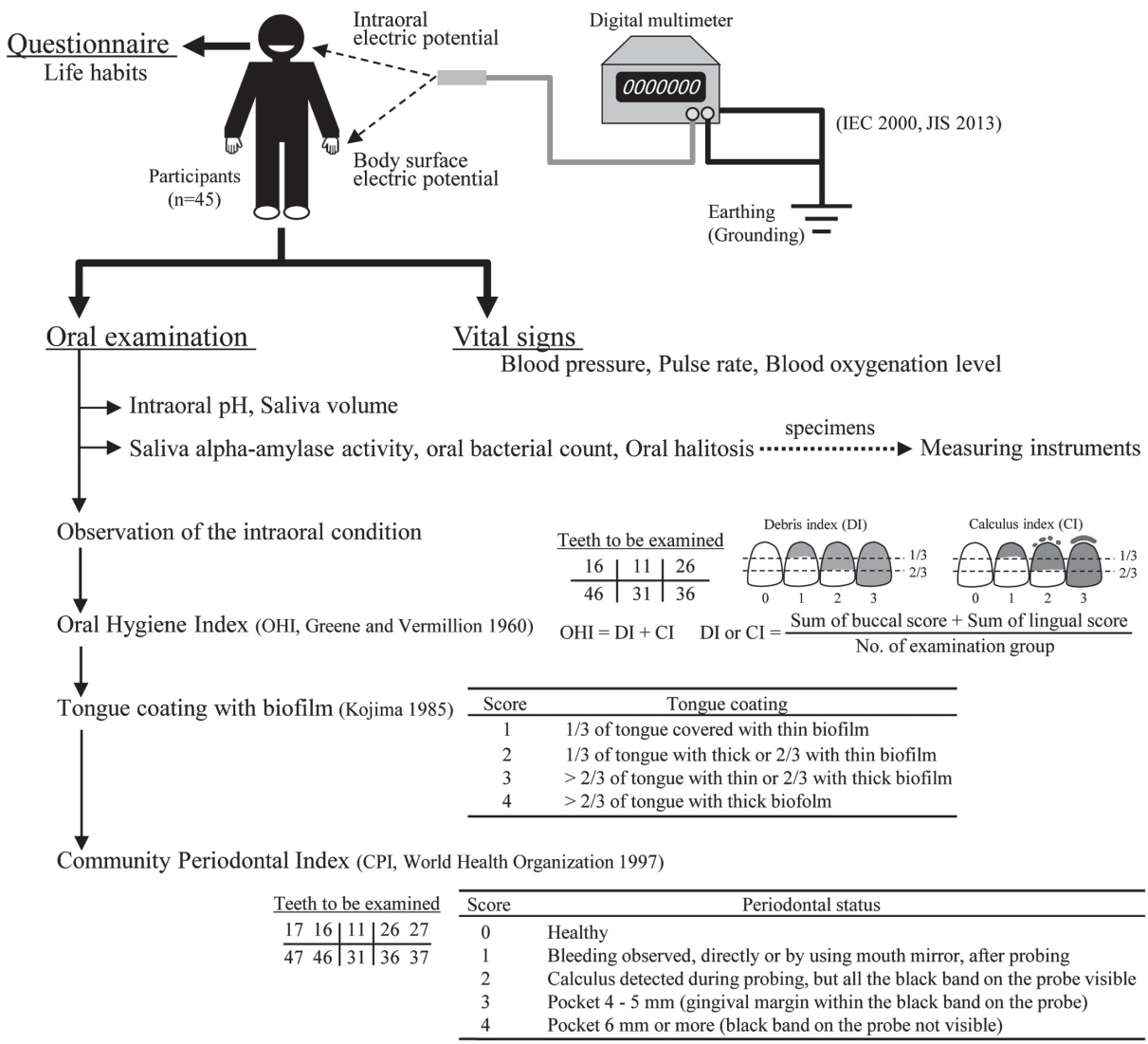


Fig. 1 Schematic representation of the flow of the *in vivo* study.

body surface during the measurement, so that the IoP/BsP values rapidly decreased to approximately 0 V (<1.0 μV). Intraoral pH was measured using pH indicator paper (Pehanon pH 6.0–8.1 ref. 904 17, Macherey-Nagel, Düren, Germany). Saliva was collected in a centrifugation tube (ECK-15ML-R, AS ONE, Tokyo, Japan) after the participant allowed saliva to collect in his mouth for 1 min, and was measured using a micropipette (Research Plus, Eppendorf, Hamburg, Germany). Salivary alpha-amylase activity as a stress marker was measured using a salivary amylase monitor (DM-3.1, Nipro, Osaka, Japan)[18]. Stress is strong so that salivary alpha-amylase activity is big, and threshold value of having stress is 30 kIU/L. Blood pressure was measured using a digital sphygmomanometer (HEM-7114 [accuracy; ±3 mmHg], Omron Health Care, Kyoto, Japan). Blood oxygenation ($SpO_2$) and pulse rate were estimated with a pulse oximeter (Pulse Fit BO-650 [accuracy; $SpO_2$: ±2%, pulse rate: ±3%], Japan Precision Instruments, Gunma, Japan). Normal value of $SpO_2$ is 96–99%. Intraoral bacterial count was measured from a specimen obtained by touching the surface of the participant's tongue with a dedicated cotton bud attached to a holding device (Constant pressure sample collection device, DU-AE01NT-H, Panasonic Healthcare, Tokyo, Japan) using a bacteria counter (DU-AA01, Panasonic Healthcare) according to the manufacturer's instruction[19,20]. Allowance range of normal bacterial count is $<1\times10^8$/mL using this measurement instrument. Oral halitosis was measured using a volatile sulfur compounds monitor (Halimeter Model RH17E [accuracy; ±5 ppb], Interscan, Simi Valley, CA, USA)[21]. Allowance range of normal oral halitosis is <300 ppb in this instruments. All measurements were performed according to the manufacturers' instructions.

Participants were then examined by one periodontist to determine their oral hygiene index (OHI)[22], degree of tongue coating with biofilm[23], and community periodontal index (CPI)[24] (Fig. 1). Each examination was performed using disposable sterilized dental instruments (Dispon series, BSA Sakurai, Nagoya, Japan) and dental CPI-probes (WHO probes) sterilized in the autoclave.

*Power generating capacity and biofilm accumulation of Streptococcus mutans using a MFC in vitro*

To evaluate the power generating capacity of *S. mutans* (JCM 5705, RIKEN BioResource Center, Ibaraki, Japan) as the main oral bacteria in oral biofilm, a two-chamber type MFC was used (Fig. 2)[25]. The fuel chamber consisted of an acrylic experimental apparatus for ion movement (PP-9, Uchida Yoko, Tokyo, Japan) with a carbon felt electrode (2.0×2.5×1.0 cm, S-222, Osaka Gas Chemical, Osaka Japan), a load resistance of 1 kΩ, and a proton exchange membrane (Nafion 117, The Chemours, Fayetteville, NC, USA) or a polyvinylidene chloride (PVDC) film (Asahi Kasei, Tokyo, Japan). The carbon felt electrode was soaked in 5 mL of *S. mutans* bacterial suspension with an optical density of 0.8 at 660 nm, and cultivated in the anode chamber solution,

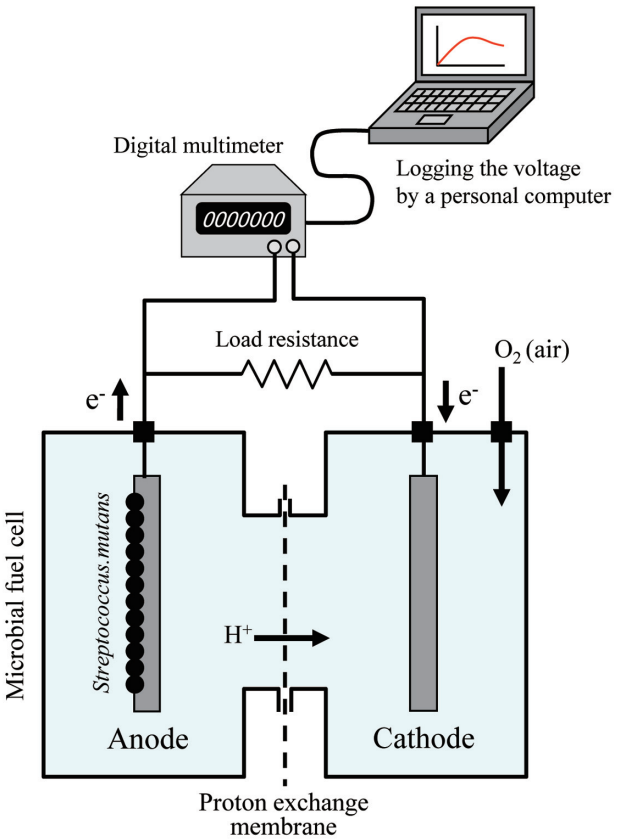


Fig. 2 Schematic diagram of the *in vitro* experiment with a two-chamber type MFC.

*i.e.* 10% brain heart infusion medium (BHI: Beckton, Dickinson, Franklin Lakes, NJ, USA), 3% sucrose, $NH_4Cl$ (0.31 g/L), $KH_2PO_4$ (4.4 g/L), $K_2HPO_4$ (3.4 g/L), NaCl (0.5 g/L), $MgCl_2$-$6H_2O$ (0.15 g/L), and $CaCl_2$-$2H_2O$ (0.15 g/L) in sterilized distilled water (measured pH value; 6.6±0.03 [range; 6.5–6.6]). The cathode chamber solution was $NaHCO_3$ (1.0 g/L), $NH_4Cl$ (0.5 g/L), $KH_2PO_4$ (4.4 g/L), $K_2HPO_4$ (3.4 g/L), NaCl (0.5 g/L), $MgCl_2$-$6H_2O$ (0.15 g/L), and $CaCl_2$-$2H_2O$ (0.15 g/L) in sterilized distilled water (measured pH value; 6.9±0.09 [range; 6.8–7.0]), which was continuously aerated by an air pump (S200, Japan Pet Design, Tokyo, Japan) during operation of the MFC. The pre- and post-experimental pH of the anode and cathode solutions were measured with a pH meter (LAQUA twin B-712, Horiba, Kyoto, Japan). The voltage generated by *S. mutans* was measured with a DMM in DC mode every 30 s, and data from the DMM were logged using a personal computer (CF-S9, Panasonic, Osaka, Japan) for 3–6 h. Maximum electric power ($P_{max}$) was calculated from the maximum voltage ($V_{max}$) and load resistance values (R), *i.e.* $P_{max}=RI^2=V_{max}^2/R$. Maximum power density was calculated as the value of $P_{max}$ divided by the surface area of the anode electrode (5.0 $cm^2$). All materials excluding the DMM and the personal computer were

sterilized in an autoclave or with ethylene oxide before experiments. The evaluation was performed in the laboratory, which was maintained at a temperature of 22.6±0.90°C [range; 21.4–23.8°C]. After evaluation, no contamination of the anode and cathode solution was observed under a microscope. Biofilm that formed on the surface of the anode electrode after 3 h in an MFC was collected in a centrifugation tube, and centrifuged for 10 min at 800×g to separate the biofilm from floating bacteria. After the supernatant was discarded, the biofilm was centrifuged for 20 min at 4,030×g. The pellets were dried in an incubator (IC-41, Yamato Scientific, Tokyo, Japan) at 70°C overnight, and the dry weight was measured as the biofilm amount using an electronic balance (AE240-S, Mettler-Toledo International, Greifensee, Switzerland) with a readability of 0.01 mg placed on a suitable mounting (Vibro-Absorbing Mount VAM-I, Murakami Koki, Osaka, Japan).

*Data processing and statistical analysis*

This was a double-blind study. Participants were allocated a number for each experiment. Questionnaire results and measurement data obtained from each measurement item performed by researchers who did not know the participants' numbers or results in other measurement items, were combined, organized and calculated by another researcher. After encryption of the item name, these data were statistically analyzed by another researcher. The data were analyzed using Spearman's correlation coefficient by rank test to determine the correlation between IoP/BsP and each examination item at a significance level of 5%. To examine the common factors, examination items were analyzed using factor analysis with a biquartimin criterion for rotation to an oblique simple structure after exclusion of items which exhibited a factor score of less than 0.3 in factors 1, 2 and 3, which exhibited more than 10% value of the contribution ratio before oblique rotation. For the *in vitro* study, each experiment was repeated eight times, and the maximum and minimum values were excluded prior to analysis to eliminate the risk of errors from outliers. The remaining six values were used to calculate the mean±standard deviation (SD). Data were then analyzed using one-way ANOVA with Bonferroni's post-test to reveal statistically significant differences between data sets. Data were also analyzed using Spearman's rank correlation coefficient test and single regression analysis to determine the correlation between Vmax/Pmax and the amount of biofilm accumulation in the MFC with a proton exchange membrane at a significance level of 5%. All statistical analysis was performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan)[26], which is a graphical user interface for R (The R Foundation for Statistical Computing; Vienna University of Technology, Vienna, Austria) on a workstation computer (MB-P5300X-WS, Mouse Computer, Tokyo, Japan).

## RESULTS

Measurement flow of each item in 45 male participants who were fifth year university students (mean age; 20.1±0.96 years, range; 19.3–23.7 years) at the National Institute of Technology, Nagaoka College was shown in Fig.1. Correlations between intraoral (IoP)/body surface electric potentials (BsP) and other examination items are shown in Table 1, and correlation diagrams and histograms are shown in Fig. 3. Items not listed in Fig. 3 and Table 1 did not exhibit significant correlation with any other examination items.

There was a high correlation between IoP and BsP (r=0.88, $p<0.01$) (Fig. 3 and Table 1). Although correlation between BsP and OHI/CPI was not observed, IoP was loosely correlated with oral hygiene (OHI; r=0.31, $p<0.05$ and DI; r=0.32, $p<0.05$) and periodontal condition (CPI; r=0.33, $p<0.05$) (Fig. 3). Correlations between OHI and CPI were observed (DI-CPI; r=0.70, CI-CPI; r=0.80, OHI-CPI; r=0.77, $p<0.01$) (Table 1). In spite of their high correlation coefficient values, the correlation diagrams shown in Fig. 3 reveal that these correlations could not be clearly determined as high correlations. Oral halitosis was not significantly correlated with oral bacterial count, OHI, CPI, or salivary alpha-amylase activity. Salivary alpha-amylase activity was loosely correlated with CPI (r=0.31, $p<0.05$), but not OHI (Fig. 3 and Table 1).

As IoP and BsP were correlated with smartphone use, the correlation coefficient of IoP (r=0.40, $p<0.01$) was higher compared with BsP (r=0.34, $p<0.05$) (Fig. 3 and Table 1). However, smartphone use, which was an external factor for IoP and BsP elevation, was not correlated with OHI and CPI. Other items relating to oral condition, life habits, and vital signs were not significantly correlated with IoP and BsP, or to OHI and CPI (Table 2). Among the above-mentioned items, no significant correlations were observed.

Factor analysis of examination items significantly correlated with other items revealed that BsP and IoP, BsP and smartphone use, IoP and OHI/CPI, salivary alpha- amylase and CI/CPI, and CPI and OHI were common factors (Fig. 4). The results of the factor analysis of all the examination items were almost the same as those of examination items significantly correlated with other items.

Using a two-chamber type MFC, the power generating capacity of *S. mutans* was evaluated (Fig. 2). Load resistance was set at 1 kΩ, which was almost the same as the internal resistance of an MFC with *S. mutans* from the results of preliminary experiments. Maximum voltage generated by *S. mutans* was 110.3±18.46 mV (range; 87.0–132.6 mV), and maximum power density was 2.5±0.81 μW/cm$^2$ (range; 1.5–3.5 μW/cm$^2$) in this MFC model (Fig. 2 and Table 3). This *S. mutans*-generated electricity was accompanied by biofilm accumulation on the anode electrode surface (Fig. 5 and Table 3). Biofilm that formed on the surface of the anode electrode after 3 h in an MFC with a proton exchange membrane (a complete fuel cell with power

Table 1 Correlation between intraoral/body surface electrical potential and each examination item

A. Measured values of examination items

| Electric potential | | Oral condition | | | | | Life habit |
|---|---|---|---|---|---|---|---|
| Body surface (BsP) (mV) | Intraoral (IoP) (mV) | DI (Debris Idx) | CI (Calculus Idx) | OHI (DI+CI) | CPI (max value) | αAMY (kIU/L) | Smartphone use (h/day) |
| 36.0±5.68 (25.1–49.8) | 35.0±5.98 (24.2–48.1) | 1.1±0.68 (0.2–1.8) | 0.2±0.16 (0–0.7) | 1.3±0.77 (0.5–3.7) | 1.4±0.83 (0–3) | 21.0±19.39 (2–97) | 3.8±2.62 (0–12) |

$n$=45 for each item. Values are mean±SD and (range).
αAMY: Salivary alpha-amylase activity

B. Correlation between intraoral/body surface electrical potential and each examination item

| | | Electric potential | | Oral condition | | | | | Life habit |
|---|---|---|---|---|---|---|---|---|---|
| | | BsP | IoP | DI | CI | OHI | CPI | αAMY | SP use |
| Electric potential | BsP | 1 | — | — | — | — | — | — | — |
| | IoP | 0.88** ($5.10\times10^{-9}$) | 1 | — | — | — | — | — | — |
| Oral condition | DI | 0.22 (0.14) | 0.32* (0.04) | 1 | — | — | — | — | — |
| | CI | 0.16 (0.29) | 0.23 (0.13) | 0.55** ($2.34\times10^{-4}$) | 1 | — | — | — | — |
| | OHI | 0.22 (0.15) | 0.31* (0.04) | 0.98** ($7.60\times10^{-11}$) | 0.68** ($6.84\times10^{-6}$) | 1 | — | — | — |
| | CPI | 0.27 (0.07) | 0.33* (0.03) | 0.70** ($3.97\times10^{-6}$) | 0.80** ($1.06\times10^{-7}$) | 0.77** ($3.84\times10^{-7}$) | 1 | — | — |
| | αAMY | −0.07 (0.66) | 0.07 (0.66) | 0.14 (0.37) | 0.25 (0.10) | 0.15 (0.32) | 0.31* (0.04) | 1 | — |
| Life habit | SP use | 0.34* (0.02) | 0.40** ($8.19\times10^{-3}$) | 0.26 (0.08) | 0.05 (0.73) | 0.24 (0.11) | 0.28 (0.07) | 0.12 (0.44) | 1 |

$n$=45 for each item. Values are correlation coefficients and ($p$ values). *$p$<0.05, **$p$<0.01.
BsP: body surface electric potential, IoP: intraoral electric potential, αAMY: Salivary alpha-amylase activity, SP: smartphone

generation) (91.4±23.69 mg [range; 58.3–121.4 mg]) produced a 35-fold increase compared with the amount produced in an MFC with a PVDC film (an incomplete fuel cell with generating capability reduced by blocking of proton transfer from the anode chamber to the cathode chamber) (2.6±1.73 mg [range; 0.7–5.0 mg]) (Table 3). The amount of biofilm accumulated in the anode chamber correlated with the *S. mutans* electric power generation in this MFC system (Figs. 5 and 6, Table 3). After the experiments, the pH of the anode and cathode solutions measured 4.8±0.14 (range; 4.7–5.0) and 6.9±0.05 (range; 6.8–7.0) in the MFC with a proton membrane, and 6.4±0.06 (range; 6.4–6.5) and 7.0±0.03 (range; 7.0–7.1) in the MFC with a PVDC film, respectively.

## DISCUSSION

To clarify the relationships between intraoral (IoP)/body surface electric potentials (BsP) and oral condition/vital signs/life habits, we examined each item in 45 participants who were similar in terms of sex (all male to exclude effects of fluctuating hormones on oral condition), age (20.1±0.96 years), and living environment (same year-grade students belonging to the same division of the same school; 60% lived in the same dormitory and ate the same school lunch). Questionnaire items about life habits revealed that their lifestyle was relatively similar in terms of hours of sleep, number of meals per day, smoking, and frequency and duration of toothbrushing. The number of decayed and filled teeth also exhibited low values and narrow range (Table 2). Uniformity of the participants in this study, especially

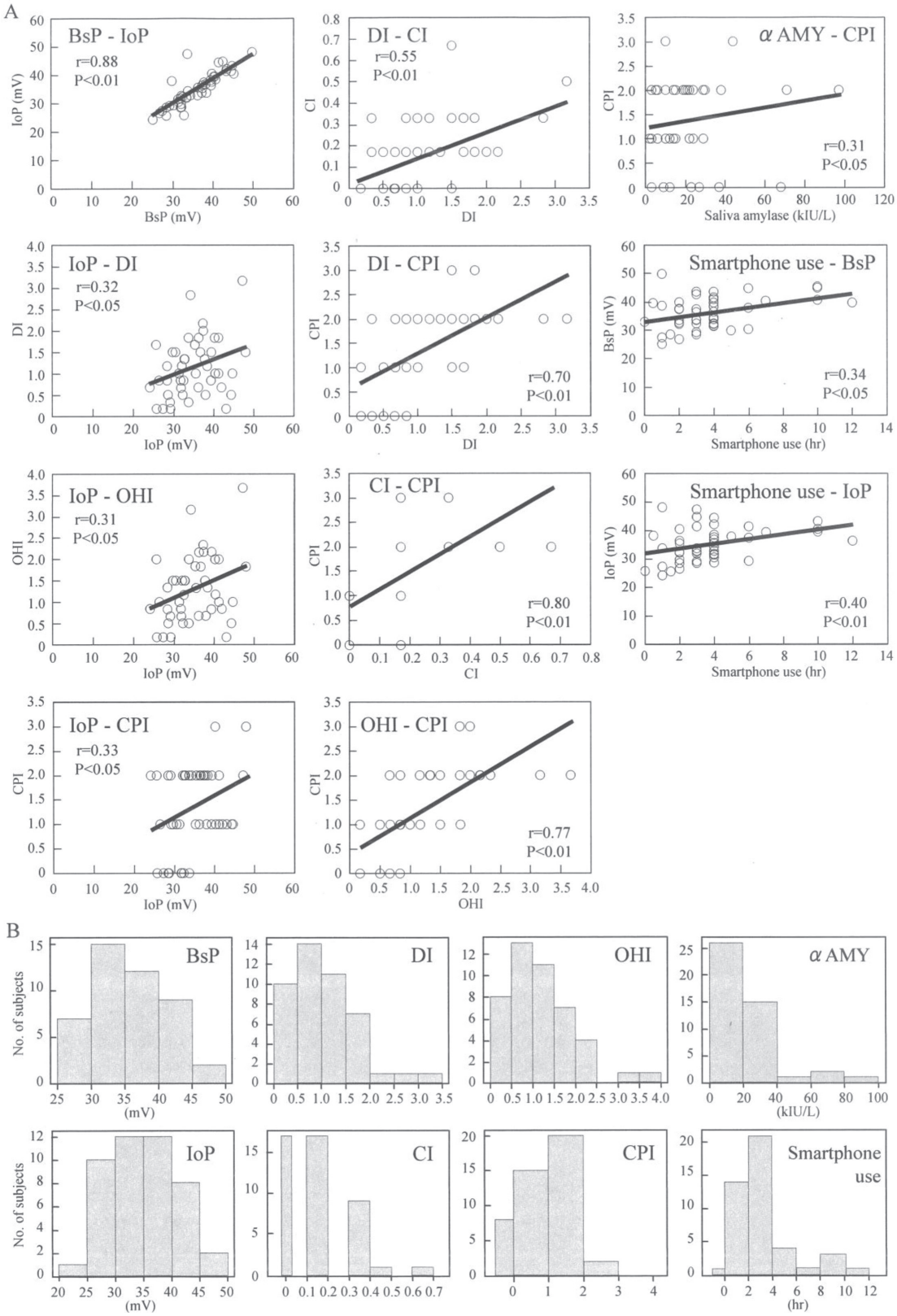


Fig. 3 Correlation diagrams and histograms of examination items significantly correlated with other items. A: Correlation diagrams of examination items. B: Histograms of examination items. BsP: body surface electric potential, IoP: intraoral electric potential, OHI: oral hygiene index, DI: debris index, CI: calculus index, CPI: community periodontal index, αAMY: saliva alpha-amylase activity.

Table 2 Measured values of examination items for oral condition, life habits and vital signs not significantly correlated with intraoral/body surface electrical potential

A. Oral condition

| | | Intraoral pH | Saliva volume (μL) | Oral bacterial count ($\times10^6$/mL) | Oral halitosis (ppb) | Decayed teeth (no. of teeth) | Filled teeth (no. of teeth) |
|---|---|---|---|---|---|---|---|
| Means±SD (range) | | 6.9±0.26 (6.0–7.2) | 527.4±347.5 (89–1,291) | 1.4±1.01 (4.4–45.5) | 133.8±102.5 (24–413) | 0.62±1.5 (0 (34 P)–6) | 1.2±2.3 (0 (31 P)–8) |
| R | BsP | $3.2\times10^{-3}$ | $7.4\times10^{-2}$ | −0.19 | $1.4\times10^{-2}$ | 0.15 | 0.20 |
| | IoP | $7.8\times10^{-3}$ | $2.8\times10^{-2}$ | −0.12 | $5.2\times10^{-3}$ | 0.20 | 0.19 |

B. Life habits

| | | Sleeping time (h/day) | Meal times (times/day) | Smoking | PC use (h/day) | TV viewing (h/day) | Toothbrushing | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Frequency (times/day) | Duration (min/day) |
| Means±SD (range) | | 5.8±0.80 (4–7) | 2.9±0.38 (2–4) | 3 light smokers* | 3.5±2.17 (0.5–8) | 1.0±1.07 (0–4) | 2.0±0.50 (1–3) | 12.0±10.10 (3–60) |
| R | BsP | 0.24 | 0.17 | na | $5.9\times10^{-2}$ | $1.2\times10^{-2}$ | 0.18 | $-8.8\times10^{-2}$ |
| | IoP | 0.19 | 0.14 | na | $8.1\times10^{-2}$ | $7.9\times10^{-2}$ | 0.14 | −0.15 |

C. Vital signs

| | | Blood pressure | | Pulse rate (beats/min) | Blood oxygen level (%) |
|---|---|---|---|---|---|
| | | Systolic (mmHg) | Diastolic (mmHg) | | |
| Means±SD (range) | | 129.1±13.63 (106–170) | 70.5±8.39 (54–100) | 73.4±15.07 (53–122) | 98.5±0.55 (98–99) |
| R | BsP | −0.10 | −0.19 | −0.11 | 0.11 |
| | IoP | $-3.0\times10^{-2}$ | $-9.5\times10^{-2}$ | $-1.7\times10^{-2}$ | 0.14 |

$n$=45 for each item. $p$ Value of correlation coefficient between IoP/BsP and each item was >0.1.
na: not available.
BsP: body surface electric potential, IoP: intraoral electric potential, R: correlation coefficient, P: participants, PC: personal computer, TV: television.
*: a few cigarettes/day

with their young age and good health, could be one of the reasons why the number of decayed and missing teeth was not related to toothbrushing frequency and duration, as well as other items relating to oral hygiene including intraoral pH, saliva volume, oral halitosis and oral bacterial count. In spite of their uniformity, many statistically significant correlation coefficient values in this study were relatively low, except those of IoP–BsP and CPI–OHI. The reason for the low values of these significant correlation coefficients could be related to variations in the participants' condition.

Most people are unaware that the atmosphere carries a continuous electric current. The electric potential increases by about 100 volts per meter from the ground up as a global electric circuit[27]. These electric potentials influence the electric charge of the human body[28]. Given that smartphone-induced IoP/BsP was observed with high correlation with IoP and BsP, smartphone use could be an external factor in IoP/BsP elevation as well as the global electric circuit *via* direct and/or indirect processes[29]. Even if the influence of these external factors on IoP and BsP is small, they should exert some influence on IoP and BsP in participants without earthing to the ground. The correlation coefficient of smartphone use-IoP was higher than that of smartphone use-BsP. Smartphone use did not correlate with OHI and CPI elevation. The electromagnetic wave produced by the smartphone is microwave range, which are used for sterilization of bacteria by its thermal effect[30]. This might be one of the reasons about no significant correlation between smartphone use and OHI/CPI. These results suggest that the electric potential of the body surface could be charged externally, which could influence the intraoral charge, and might be more easily discharged than the intraoral charge by touching electro-conductive

A

| | Contribution ratio (%) | Cumulative ratio (%) |
|---|---|---|
| Factor 1 | 39.8 | |
| Factor 2 | 18.5 | 58.3 |
| Factor 3 | 10.3 | 68.6 |
| Factor 4 | 4.6 | 73.2 |
| Factor 5 | 2.0 | 75.2 |

B

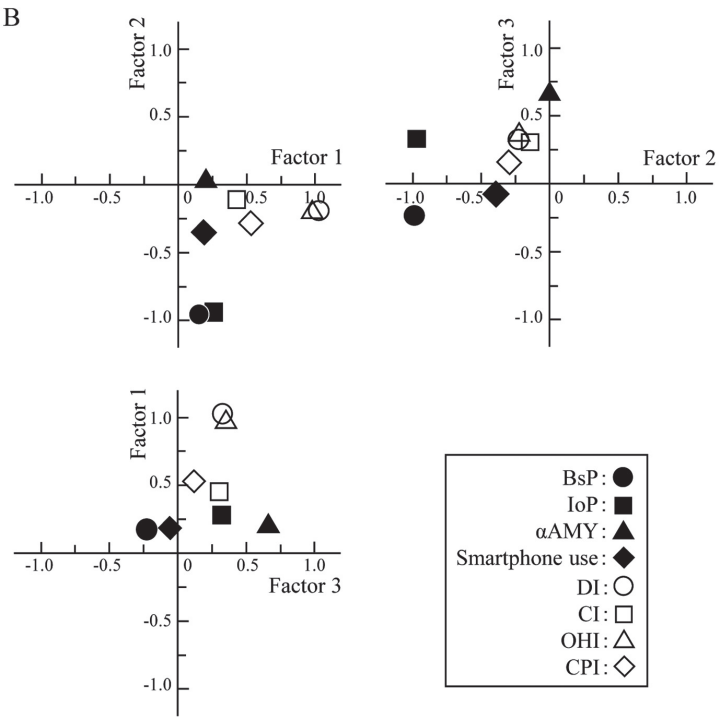


Fig. 4 Factor analysis of examination items significantly correlated with other items. A: Contribution ratio before biquartimin rotation. B: Scatter plots of the factor analysis after biquartimin rotation. BsP: body surface electric potential, IoP: intraoral electric potential, OHI: oral hygiene index, DI: debris index, CI: calculus index, CPI: community periodontal index, αAMY: saliva alpha-amylase activity.

Table 3 Generating capacity and biofilm formation of *Streptococcus mutans* using a MFC

| Anode chamber (electrode surface) | Membrane | Maximum voltage (mV) | Maximum power density (μW/cm$^2$) | Biofilm on anode electrode (mg) | Anode solution pH after exp. |
|---|---|---|---|---|---|
| None (medium only) | Proton exchange membrane | $0.6\pm0.08\times10^{-1}$ ($0.5–0.7\times10^{-1}$) | $2.6\pm0.85\times10^{-7}$ ($4.8–9.0\times10^{-7}$) | na | 6.7±0.02 (6.6–6.7) |
| *Streptococcus mutans* | Polyvinylidene chloride film | $0.3\pm0.05\times10^{-1}$ ($0.3–0.4\times10^{-1}$) | $6.8\pm1.83\times10^{-7}$ ($1.9–3.9\times10^{-7}$) | 2.6±1.73 (0.7–5.0) | 6.4±0.06 (6.4–6.5) |
| *Streptococcus mutans* | Proton exchange membrane | 110.3±18.46 (87.0–132.6) | 2.5±0.81 (1.5–3.5) | 91.4±23.69 (58.3–121.4) | 4.8±0.14 (4.7–5.0) |

** ** ** † †

$n$=6, **$p$<0.01, †significant correlation by Spearman's rank correlation coefficient test ($p$<0.05)
na: not available, exp: experiment

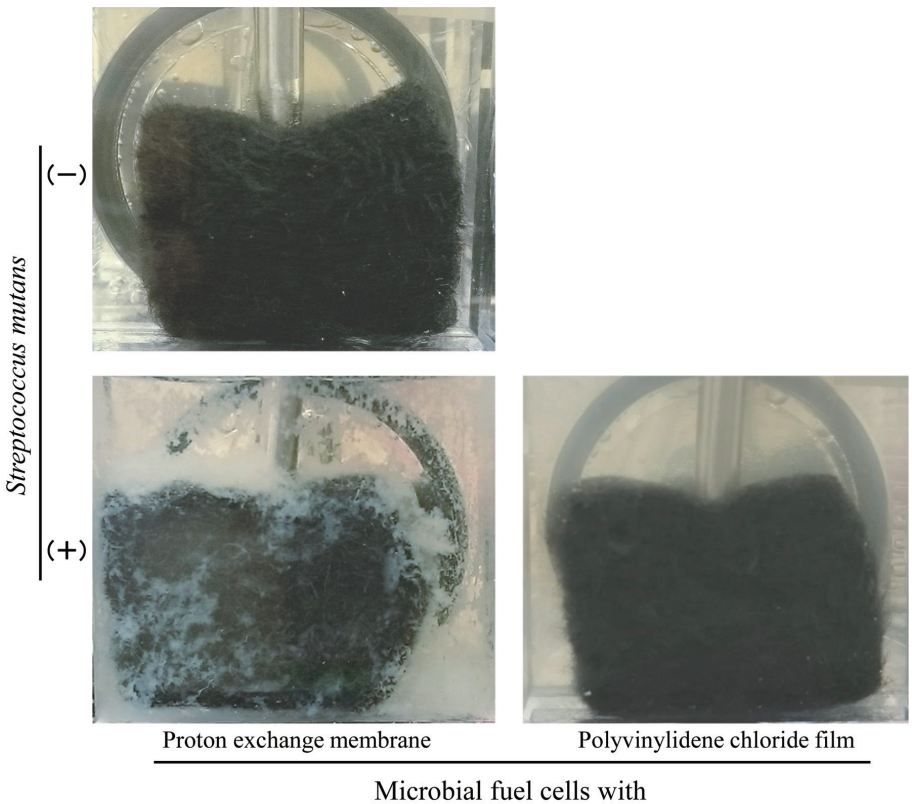


Fig. 5 *In vitro* biofilm formation of *Streptococcus mutans* in the anode chamber of a MFC after 3 h operation.

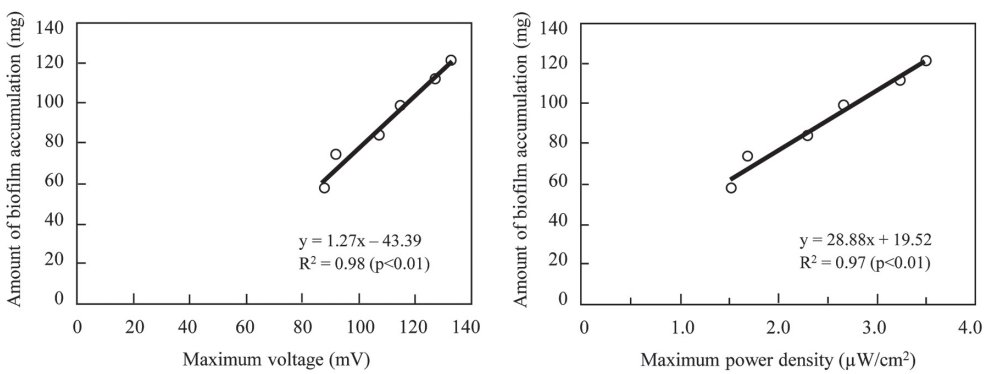


Fig. 6 Regression functions and scatter plots of the power generating capacity and biofilm accumulation of *Streptococcus mutan*s in a MFC.

materials. Interestingly, television viewing and personal computer use did not correlate with IoP/BsP. This may be because of the increased distance of these devices from the participants. Another possible reason is that users usually operate smartphones in their hands, directly touching capacitive type touch panels, and with their fingers involved in the electric field[31].

Our results reveal that IoP was correlated with oral hygiene (OHI) and periodontal condition (CPI), but BsP was not. Taking into consideration the results of smartphone-induced IoP/BsP, oral conditions could influence IoP, rather than IoP influencing the oral conditions. These causal relationships could be involved in a mechanism similar to the oxidation–reduction potential of oral bacterial flora in the process of microbiologically influenced corrosion[32] and MFC[25].

In this study, the participants were a relatively uniform group. From the distribution of CPI values and the young age of the participants, the CPI values in this study could be correlated with gingivitis rather than periodontitis (Fig. 3). This could be one reason why OHI exhibited significant correlation with CPI with a high correlation coefficient. Oral halitosis was not correlated with oral bacterial number, and neither oral halitosis nor oral bacterial number were correlated with OHI and CPI. Specimens for the oral bacterial count were obtained from the surface of participant's tongue using a dedicated cotton bud[20]. Tongue coating acts as a direct and primary source of oral malodor, which has been extensively documented[33,34]. The lack of a significant correlation between tongue coating level and OHI/CPI could correspond with the absence of a significant correlation between oral halitosis/oral bacterial count and OHI/CPI.

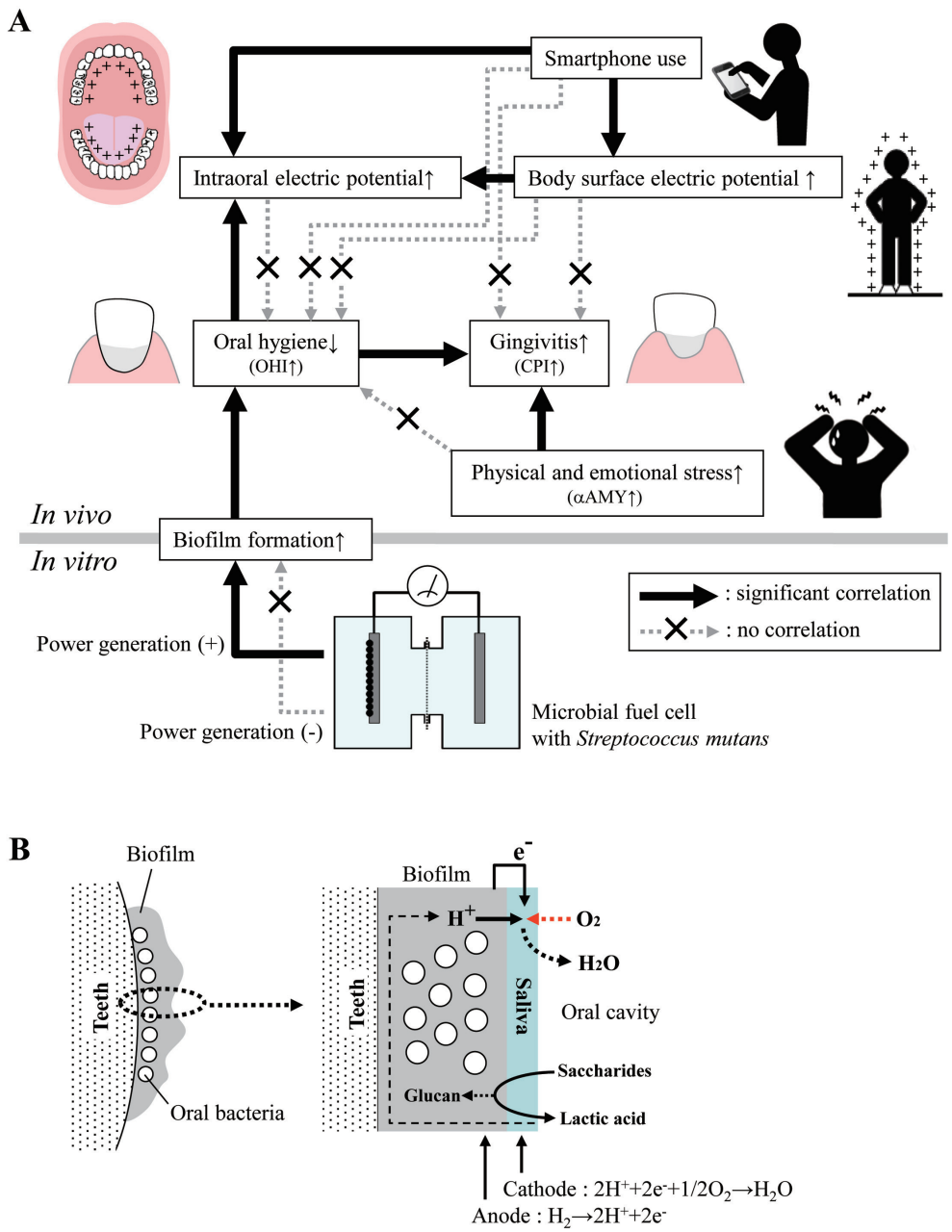


Fig. 7 Schematic representation of summary of this study.
A: Summary of the results of this study. B: Deduced model of an oral MFC. OHI: oral hygiene index, CPI: community periodontal index, αAMY: saliva alpha-amylase activity.

The examination items relating to vital signs revealed no significant correlation between each vital sign and IoP/BsP or OHI/CPI. Additionally, no vital item had a significant correlation with any item relating to life habits. No significant correlation was observed between any life habit item and IoP/BsP, apart from smartphone use (Fig. 3 and Table 1). These findings may have resulted in part from the uniformity of the participants in this study.

Salivary alpha-amylase activity is a reflection of physical and emotional stress[18]. There is little variation caused by aging, but daily fluctuations occur in salivary alpha-amylase activity[35,36]. For this reason, this examination was performed at the same time each day (15:00 to 17:00). Elevation of salivary alpha-amylase activity correlated with elevation of the CPI value, but not the OHI value. Periodontal disease is reported to relate to physical and emotional stress[37]. The results of our study suggest that stress could have made the periodontal condition worse in a stressed participant, even if their oral hygiene was good. Moreover, salivary alpha-amylase activity was not significantly correlated with IoP and BsP. This suggests that stress in the participants could have made their periodontal condition worse, independent of IoP and BsP elevation. In contrast, smartphone use should elevate IoP and BsP, which was not related to oral hygiene and periodontal condition. This indicates that the electric potential of the body surface and oral cavity could not be direct causal factors for biofilm formation and periodontal disease.

Factor analysis revealed common factors among the examination items, which indicated that BsP and IoP, BsP and smartphone use, IoP and OHI/CPI, salivary alpha-amylase and CI/CPI, CPI and OHI were common factors. These results support and confirm the results of correlation analysis with Spearman's rank correlation coefficient.

To clarify the relationships between IoP and OHI, we examined the generating capacity of *S. mutans* using a MFC. *S. mutans* was selected from indigenous oral bacteria in this study because: (1) it is one of the main bacteria in oral biofilm; (2) it is suitable for an acidic environment; (3) it is an acid-producing bacterium; (4) it is a facultative anaerobe; and (5) it can form biofilm easily in an *in vitro* study[38-40]. From this *in vitro* experiment, the power generating capacity of *S. mutans* accompanied by biofilm accumulation was confirmed using an MFC with a proton exchange membrane with a substantial decrease in the pH of the anode solution (Figs 5 and 6, Table 3). In contrast, biofilm accumulation and electricity generation was blocked by the MFC with a PVDC film which was incapable of proton transfer from the anode chamber to the cathode chamber, but was possible with a slight decrease in the pH of the anode solution (Fig. 5 and Table 3). Biofilm forming at the anode electrode surface has been reported to possess electrical conductivity[41], and the biofilm of *Geobacter sulfurreducens* has been shown to possess not only electrical conductivity, but also the ability to accumulate electricity[42,43]. Results from this *in vitro* study and these reports suggest that IoP elevation is caused by biofilm accumulation induced by power generation of oral bacteria, resulting in elevation of OHI. Further studies should be conducted to investigate in detail the power generating capacity of other oral bacteria involved in oral biofilm formation.

In this study, oral electric potential was significantly correlated with BsP. BsP could be influenced by external factors, such as smartphone use. In contrast, IoP could be influenced not only by external factors, but also by the BsP and internal factors including biofilm formation on tooth surfaces. This *in vitro* study revealed the power generating capacity of *S. mutans* accompanied by biofilm accumulation using a MFC. The results of *in vivo* and *in vitro* experiments in this study and a deduced oral MFC model are summarized in Figs. 7A and B, and suggest that IoP may be not only an important factor in biofilm accumulation by oral bacterial power generation, but also a possible parameter for oral hygiene assessment (Figs. 6, 7A and B). These findings may shed new light on our understanding of the mechanism of biofilm accumulation in the oral cavity. Further *in vivo* and *in vitro* studies are needed to clarify the detailed mechanisms of IoP elevation induced by oral bacteria, and biofilm formation induced by oral bacterial power generation. Future research could be applied in preventive dentistry to achieve better oral hygiene and health, improved quality of life and an extended healthy life expectancy.

## CONCLUSION

The results of the current study showed that the IoP is correlated with oral hygiene and periodontal condition, and *S. mutans*, one of the main indigenous oral bacteria, possesses power generating capacity accompanied by biofilm accumulation. We conclude that elevation of IoP could be caused by poor oral hygiene resulting from biofilm accumulation *via* oral bacterial power generation.

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## CONFLICT OF INTEREST

The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

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